Fundamentals and Applications of Supercritical Fluid Extraction in Chromatographic Science

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Abstract

The unique properties of supercritical fluids have prompted their use for a variety of applications in the field of analytical chemistry. Perhaps the most widely cited use of these compressed fluids has been in the field of chromatography, either as mobile phase eluents or as extraction solvents. This study examines the various modes in which supercritical fluid extraction (SFE) can be employed by the chromatographer. Extraction, solubilization, and fractionation conditions are predicted by the application of well-known solution thermodynamic principles. Experimental results are reported for the removal of lipid phases from natural products and the coextraction of pesticide moieties. Finally, a method of predicting the required mobile phase pressures for solubilizing and fractionating oligomeric mixtures in supercritical fluid chromatography is compared with literature data.

Introduction

Supercritical fluids (SF) are finding wide acceptance in a number of analytical disciplines as unique solvation media. By far the largest number of applications occur in the field of chromatography, where these dense gases are employed as extraction solvents and interactive mobile phases. Historically, supercritical fluid chromatography (SFC) has its origins in the mid-1960s (1–3), while its extraction analogue has only recently seen application in the field of analytical chemistry. In truth, supercritical fluid extraction (SFE) plays a mechanistic role in SFC, where it contributes to the separation of the solutes that are injected into the chromatographic system.

The majority of reported analytical chromatographic applications of SFE to date have been concerned with the coupling of SFE, using very small extraction cells, with capillary (4,5) and packed column (6,7) SFC instruments to effect sequential SFE–SFC separation schemes. Examples of these applications are reported by other contributors to this volume and will not be discussed here. The purpose of this paper is to provide a more general guideline for the application of supercritical fluids to a variety of problems facing the chromatographer. These will include sample preparation prior to chromatography, the selec-

tion of physical conditions for extraction, and the specification of chromatographic conditions needed for fractionating oligomeric mixtures.

There are a number of modes by which SFE can be applied to the preparation of samples for chromatography. These methods are illustrated in Figures 1 and 2, where an analyte is contained in a matrix of interfering components. Perhaps the simplest form of SFE is shown in Figure 1a, whereby the analyte of interest is separated from the interfering matrix components. Such an extraction case is relatively rare for reasons that will be explained later. In fact, SFE is basically not a very selective extraction method, except in cases where selective solubilization of components can be effected over a very narrow pressure range or where the solutes to be separated differ significantly in their respective physical properties (molecular weight, polarity).

Figure 1b is perhaps more generally encountered in applying SFE to sample matrices. Here the analyte of interest is coextracted with a number of interfering components. Initially, this method may not seem to be an attractive choice; however, the use of a nontoxic compressed gas as an extraction solvent offers many advantages over conventional liquid organic solvents in terms of disposal and exposure of laboratory personnel to the extracting medium. An example of this approach in pesticide residue analysis will be cited later.

An extension of Figure 1b is shown in Figure 1c. Here the analyte of interest and the interfering components are extracted by a supercritical fluid and the analyte is subsequently analyzed by an appropriate instrumental technique. The analysis may be off-line or on-line, depending on the chosen analytical method. Several examples of on-line SFE coupled with SFC (8–10), gas chromatography (11,12), or high-performance liquid chromatography (13) have been reported.

More elaborate methods are shown in Figure 2, where SFE is coupled with sorbent technology. Figure 2a proposes SFE extraction of both analyte and interfering components followed by fractionation of the analyte from interfering solutes. Application of sorbent columns for these separations may be by traditional methods after the high pressure extraction step or by switching the supercritical-fluid-derived extract on-line to a sorbent column held at an elevated pressure. Retention characteristics of compounds on selected sorbents in the presence of supercritical carbon dioxide have been reported by the author (14) and may serve as a basis for choosing conditions to isolate specific analytes.

An alternative to Figure 2a is illustrated in Figure 2b, whereby the interfering coextracted components from the SFE step are permanently isolated on the sorbent cartridge. Such a scheme can be effected with a supercritical fluid medium throughout both the extraction and isolation steps. The analyte can then be directly introduced into the chosen instrumental technique. Such a method has been recently reported for fractionating carbamate pesticides from coextracted lipid components (15).

Finally, it should be recognized that supercritical-fluid-based analytical techniques, such as chromatography, offer the analyst exciting possibilities for eliminating sample workup techniques completely. The author has reported several examples of direct injection of complex matrices into a supercritical fluid chromatograph, thereby effecting the separation of the analyte from the interfering components without resorting to any formal extraction step before analysis (16). Such a method, Figure 2c, makes use of SFE as an in situ step during the SFC process. An example of Figure 2c will be provided later.

Theory

Many theoretical approaches for predicting the solubility and phase equilibria of solutes in supercritical fluid solvents have been reported (17–21). These theories require an array of physicochemical data and considerable time to yield information that is pertinent to optimizing extraction conditions. Such methods are of limited value to the chromatographer faced with day-to-day analytical decisions and do not lend themselves to predicting the extraction parameters required for SFE or SFC of structurally complex solutes.
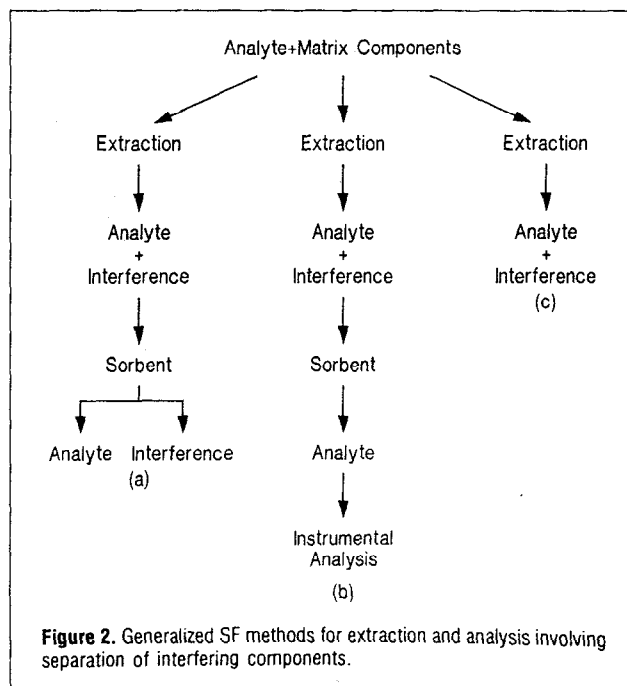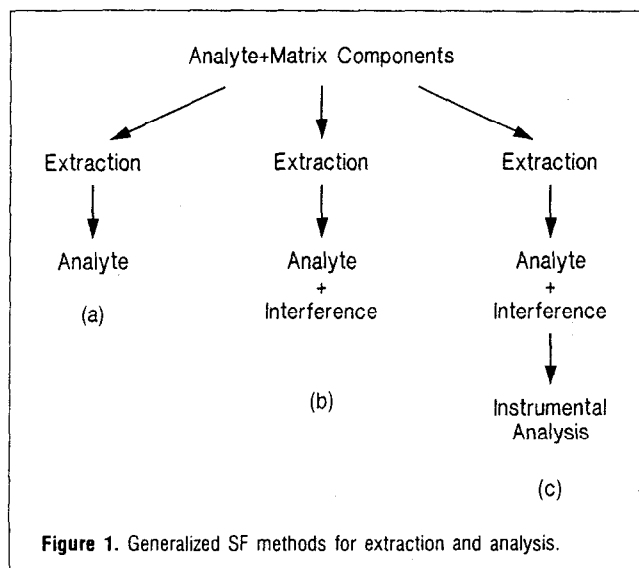
We have found that a knowledge of four basic parameters of supercritical fluid extraction are extremely helpful in understanding solute behavior in compressed gas media. The first of these parameters is the miscibility pressure, which is the pressure at which the solute starts to dissolve in the supercritical fluid. This parameter was termed the "threshold pressure" by Giddings (22) and corresponds to the critical loci of mixing between the dissolved solute and the solvent gas. As noted by the author (23), the miscibility pressure is technique-dependent and

will vary slightly, depending on the sensitivity of the analytical method that is chosen to monitor the solute concentration in the supercritical fluid phase. Nonetheless, an approximate knowledge of this pressure (or corresponding density) is very useful, for it permits the analyst to choose a starting pressure for supercritical-fluid-based fractionation processes.

Another useful parameter for specifying supercritical fluid extraction conditions is the pressure at which the solute attains its maximum solubility in the compressed fluid. This condition can be approximated by Giddings' equation which relates the solubility parameter of the gas to its critical and reduced state properties (3). When the solubility parameter of the extracting fluid (gas) is equivalent to that of the solute, maximum solubility should be attained. Solubility maxima for supercritical fluid-solute systems have been recorded by a number of investigators (24–27) and correlated by the basic tenets of the regular solution theory by King (28,29).

The third parameter, the pressure region between the miscibility and solubility maximum pressures, is the fractionation pressure range in which a solute's solubility will range between zero and its maximum value in the supercritical gas. In this interval, it becomes possible to regulate the solubility of one solute relative to another in the supercritical fluid. Enrichment of one component over another is possible by employing the variable of pressure, but it is extremely rare in SFE experiments to isolate one component from the other without resorting to an auxiliary technique (thermal gradients, chromatography, etc.). A fractionation between solutes is maximized in this pressure region by differences in the physical properties of the dissolved solutes and by keeping their concentrations low in the compressed fluid.

Finally, a knowledge of the solute's physical properties is critical to optimizing an SFE. The melting point of the solute is a particularly germane parameter in SFE, because most solutes are dissolved to a greater extent in the supercritical fluid medium when in their liquid state. Increasing the extraction temperature may also cause enhanced solute solubility in the SF because of a decrease in the solute's cohesive energy density, δ^2, and therefore its solubility parameter, δ. Hence, increasing the ex-

Figure 1. Generalized SF methods for extraction and analysis.

Figure 2. Generalized SF methods for extraction and analysis involving separation of interfering components.

traction temperature will not necessarily result in a lower solute solubility in the critical fluid.

The use of the solubility parameter theory coupled with the Flory–Huggins interaction parameter concept explains many of the phenomena encountered in supercritical fluid extraction (30–32). This approach was first utilized in polymer chemistry to predict phase miscibility relationships between polymers dissolved in dense gases, such as ethylene (33,34). The data required by the above theories consists of critical property data and solute or solvent solubility parameters. Such data is usually available or can be estimated from corresponding states theory, group contribution methods, or nomographs.

With these parameters in hand, one can use the following equation to predict the pressure at which maximum solute solubility will be attained in the supercritical fluid:

$$\chi = \chi_H + \chi_S = \overline{V}_1(\delta_1 - \delta_2)^2/RT + \chi_S \qquad \text{Eq. 1}$$

where χ is the total interaction parameter; χ_H and χ_S are the enthalpic and entropic interaction parameters, respectively; δ_1 is the solubility parameter of the gas as $f(T,P)$; δ_2 is the solubility parameter of the solute as $f(T,P)$; $\overline{V}_1$ is the molar volume of the gas as $f(T,P) = M_1/\varrho_1$; M_1 is the molecular weight of the gas; and ϱ_1 is the density of the gas. Assuming χ_S has a constant value defined by the lattice coordination number, the maximum in solubility should be achieved when δ_1 equals δ_2. Plots of χ versus pressure are hyperbolic, the minimum occuring at a value equal to χ_S. Solubility parameters for the compressed gas are calculated by

$$\delta_1 = 1.25 \, P_c^{\frac{1}{2}} \, (P_r, \, SF/P_r, \, \text{liquid}) \qquad \text{Eq. 2}$$

where P_c is the fluid critical pressure; P_r, SF is the reduced density of the supercritical fluid; and P_r, liquid is the reduced density of the fluid in the liquid state. Reduced densities can be computed from actual density data or from corresponding states relationships. The reduced densities of fluids in their liquid state are computed at infinite pressure and the proper reduced temperature from corresponding states principles.

Solubility parameters for the dissolved solutes are available from a number of literature sources (35), or they may be computed from group contribution methods if their molecular structures are known (36,37). Failure to correct for the effect of temperature on the solubility parameter of the solute can lead to a considerable error in predicting the pressures at which the solute becomes miscible with the supercritical phase or, likewise, the degree of compression required to maximize solute solubility in the dense fluid. These adjustments to δ_2, in the absence of actual experimentally derived values, can be made through the use of thermal expansion coefficients or nomographs (38). Inverse gas chromatography measurements also permit the temperature dependence of δ_2 to be determined for polymeric species (39).
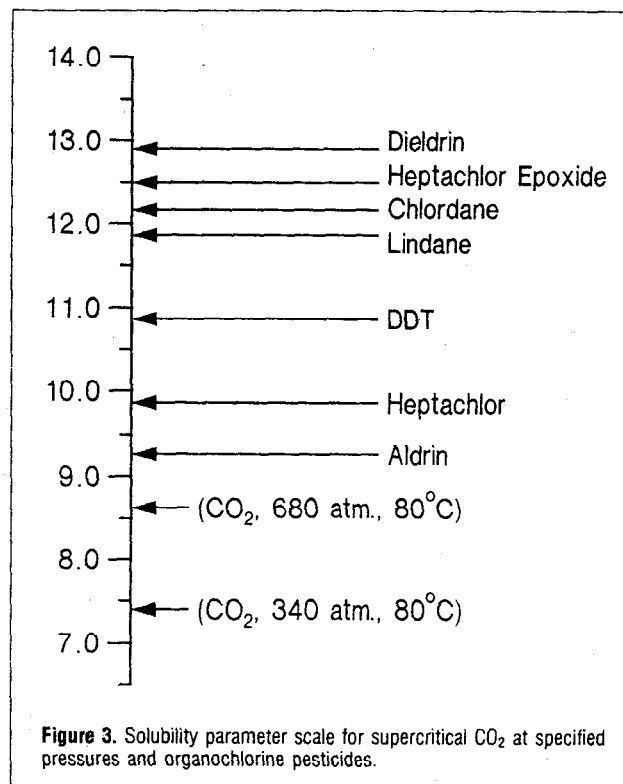
It should be noted that a 60°C temperature change can alter the δ_2 of a solute from 1.0 to 1.5 cal$^{1/2}$/cm$^{3/2}$ units (40). This variation in δ_2 might seem to be of little consequence; however, a considerable degree of fluid compression can be saved by lowering the solubility parameter of the solute to match that of the extracting fluid. In practice, both δ_1 and δ_2 are affected by temperature, but the reduction in δ_2 with temperature may more than offset the loss in δ_1 for the solvent gas. As noted before, phase changes in solute molecules may lead to significant reductions in δ_2 and their attendant molar volumes.

A common misconception in applying the above theory to SFE is that the extraction conditions must be chosen to achieve maximum solute solubility in the extracting fluid. This criterion is true if one is using SFE for the removal of a large amount of solute, such as a bulk lipid phase. However, for the removal of a trace amount of analyte from a sample matrix, much lower pressures and δ_1's will suffice. This relationship between δ_1 and δ_2 is illustrated for several organochlorine pesticides and two compression levels of supercritical carbon dioxide in Figure 3. As we shall show later, δ_1's much less than the tabulated δ_2 for the pesticides in Figure 3 are more than sufficient for dissolving trace levels of these particular analytes. Caution should be applied in introducing too large a difference in δ_1 and δ_2, because for very polar solutes, the expected solubility levels will be extremely low and perhaps beyond the detection level of many analytical techniques.

Assessment of the miscibility pressure of a solute in the supercritical fluid phase can be approximated by employing the Flory critical interaction parameter concept (41). Here, χ_c, the critical interaction parameter, is given by Equation 3 as

$$\chi_c = (1 + x^{1/2})^2/2x \qquad \text{Eq. 3}$$

where x is V_2/V_1; $\overline{V}_2$ is the molar volume of the solute $f(T,P) = M_2/\varrho_2$; M_2 is the molecular weight of the solute; and ϱ_2 is the density of the solute. In the special case of high polymer solutions in condensed liquid solvents, χ_c is 0.5 for an infinite molecular weight polymer. For our purposes, χ will change as the ratio of the molar volumes of solute and solvent are varied. Also, χ_c will vary slightly as a function of pressure, because x is particularly dependent on the variation of the molar volume of the gaseous solvent. Table 1 illustrates the possible range of χ_c values as a function of x. For the case of a high molecular weight polymer in a supercritical fluid solvent, $x = 10^4$, and

Figure 3. Solubility parameter scale for supercritical CO$_2$ at specified pressures and organochlorine pesticides.

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χ_c approaches the limiting value predicted by Flory (41). For the situation pertaining to gas–gas immiscibility ($x = 10^{-3}$), χ_c becomes quite large.

If the variations in χ (or χ_{11}) and χ_c as a function of pressure are plotted, they will intersect at a pressure corresponding to the miscibility pressure for the chosen supercritical fluid–solute system. As will be shown later, these miscibility pressures are a function of temperature and are relatively insensitive to the value one assumes for χ_s. Fractionation of one solute from another based on pressure-induced solubilization into the supercritical fluid phase is regulated by their intersection points on each solute's χ and χ_c versus pressure loci. These differences in solubility behavior can occur over relatively narrow pressure intervals and therefore require experimentally precise pressure control to effect solute fractionation.

Experimental

Several different types of experimental supercritical fluid extractions were performed to verify the predictions made by the above theories. Solubility measurements were made on a natural lipid product, soybean oil, in compressed CO_2 over an extended pressure range to test the validity of the solubility maximum and miscibility pressure concept. The apparatus employed was fabricated in our laboratories with commercially available components. Figure 4 depicts the device, which utilizes a gas booster

pump or compressor (C) to compress fluid from a compressed gas cylinder (A). Gas was introduced into the extraction cell held in a chromatographic oven by valves (SV-1 and SV-2 in tandem with SV-3 or SV-4). Soybean flakes were placed in the extractor vessel from which the solubilized oil was extracted and precipitated into a receiver vessel held slightly above room temperature by a controlled heating tape. The reduction in gas pressure within the receiver, which was held at atmospheric pressure, resulted in total separation of the dissolved triglycerides from the gaseous phase. The total quantity of carbon dioxide used to complete an extraction was quantified by a dry test meter (GT). The extracted triglycerides were measured gravimetrically by emptying the receiver at the end of the extraction. The reader is referred to several references (42–44) for further details.

Extraction experiments were also performed on porcine fat spiked with selected organochlorine pesticides to study the behavior of a trace analyte (solute) in a background matrix undergoing SFE. The apparatus described above was employed for several of these experiments, in which the extraction pressure was gradually increased in increments to allow measurement of the extracted trace component relative to the background matrix (lard). Sequential samples of the precipitated lipid phase were taken after each extraction at a particular pressure level by a valve-collection tube arrangement mounted on the receiver vessel.

Total lipid extraction of several lard samples containing spikes of a multiple pesticide mixture were made with a commercial SFE unit from Milton Roy Corporation. This Laboratory Methods Development System consisted of a CO_2 feed provided by a subambient cooled liquid chromatographic pump from a siphon tube cylinder, an extraction vessel (55 mL capacity), and several collector vessels, which could be held at a specified collection pressure by back pressure regulators. Extractions performed on this system were limited to 5000 psig (340 atm), as opposed to the laboratory system described above (Figure 4), which provided extraction data at pressures up to 12,000 psig. Extractions on the commercial instrument were performed at 5000 psig and 60°C, collection taking place at approximately 200 psig and 60°C. Lipid extracts were collected from the first

Table I. Effect of Molar Volume Ratio (x) on χ_c Value

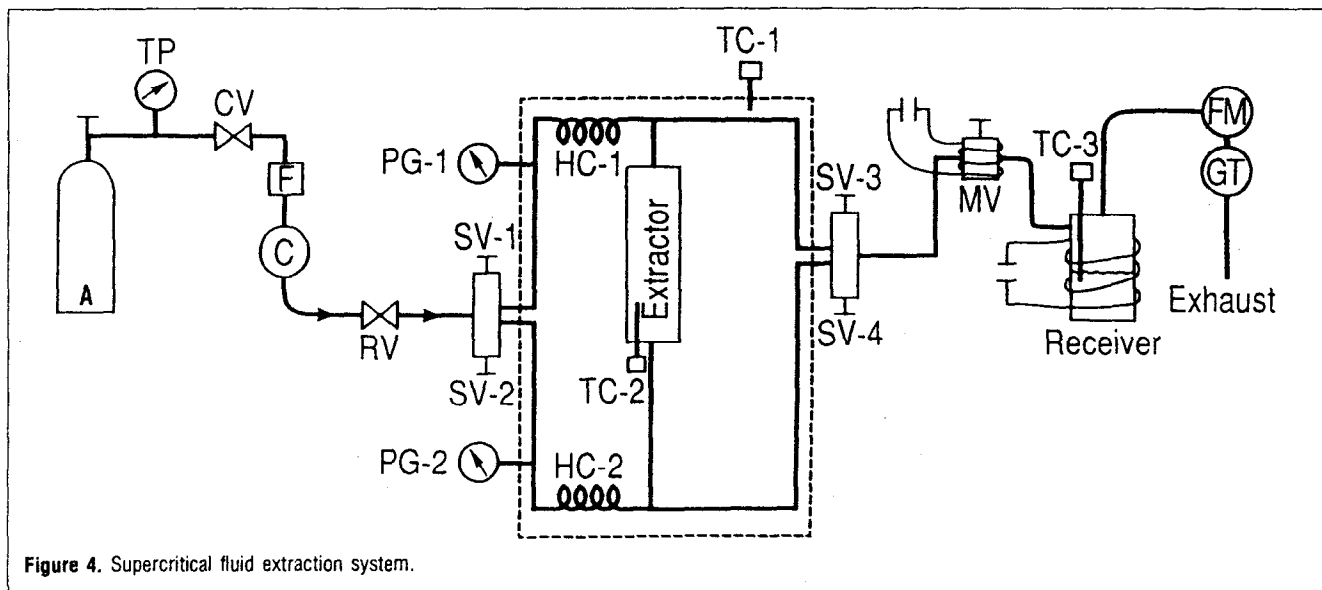| x	χ
10^4	0.510
10^2	0.065
10	0.866
1	2.000
0.5	2.910
10^{-1}	8.660
10^{-2}	60.5
10^{-3}	532

Figure 4. Supercritical fluid extraction system.

receiver vessel by opening a valve at the end of the extraction. Total lipid extractions performed on this commercial system could take several hours, depending on the sample size. This is considerably longer than the same operation performed on the laboratory-built extractor and is due in part to the lower CO_2 flow rate available from the liquid pump of the Milton Roy unit (ca. 1.3 L/min); the gas booster pump generated flows up to 20 L/min.

Analyses of the chlorinated pesticide content of the extracted lipid samples were performed by a standard sample cleanup procedure followed by electron capture–packed column GC analysis (ECD–GC) (45). Fat moieties were fractionated from the solventless extracts on a microalumina column, using a dye (*p*-methoxyazobenzene) to estimate the elution volume of the pesticide fraction. Extracts were injected into a 1.83-m × 4-mm i.d. packed column (50/50 mix of 3% QF-1 and 1.5% OV-17 + 1.95% QF-1 on 80/100 mesh GCQ) after concentration in Kdurna–Danish tube. A Hewlett-Packard model 5710 gas chromatograph equipped with a ^{63}Ni electron capture detector was used to perform the analysis. Injector and column temperatures were 200°C while the detector was held at 350°C. Aldrin was added as an internal standard in the analysis, and all quantitative results were corrected for recovery and blank.

Results and Discussion

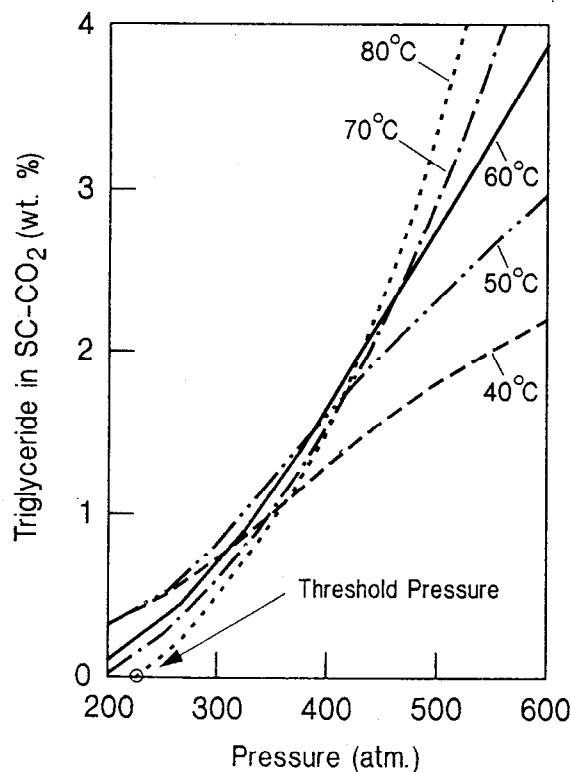Extraction results for the removal of triglycerides from soybean meal are presented in Figure 5. Here the total triglyceride content in the supercritical fluid, expressed as weight percent in the solvent gas, is plotted as a function of extraction pressure. Within the temperature and pressure range studied, there is always an observed increase in triglyceride solubility with increasing extraction gas pressure. An inversion in the temperature dependence of triglyceride solubility is noted in the range of 250–400 atm. Over this pressure interval, the solubility of extracted triglycerides increases with both temperature and pressure, the inverse of that observed at the lower pressures.

Maxima in triglyceride solubility versus pressure plots have been noted by the author (46) at much higher pressures than those given in Figure 5. The occurence of such maxima correlates very well with the relative ratio of the solubility parameters of the supercritical fluid to that of the dissolved solute, maximum or complete solubility being attained when the ratio is unity. Triglycerides become infinitely soluble in supercritical CO_2 at pressures above 950 atm and 80°C; hence, extraction of bulk lipid matter is readily facilitated under these conditions.

Initially, one would not expect higher triglyceride solubilities in supercritical CO_2 to occur as the extraction temperature is increased because of a decrease in gas density with temperature and a concomitant decrease in the solubility parameter of CO_2. However, independent measurements on soybean oil show a decrease in the solubility parameter from 8.16 $cal^{1/2}/cm^{3/2}$ at 40°C to 7.57 $cal^{1/2}/cm^{3/2}$ at 80°C (40). For reference purposes, the solubility parameter of CO_2 at 40°C and 300 atm is 8.00 $cal^{1/2}/cm^{3/2}$ and 7.06 $cal^{1/2}/cm^{3/2}$ at 80°C and 300 atm. This reduction in δ_1 and a corresponding decrease in δ_2 with increasing temperature provide an enhancement of the triglyceride solubility at elevated temperatures and pressures.

The data in Figure 5 also allow an approximation of the miscibility pressure for the soybean triglyceride–supercritical CO_2 system. Extrapolation of the data to zero solubility yields values 109 and 225 atm at 40 and 80°C, respectively. To compare these values with the results predicted by equations 1 and 3, we have calculated χ and χ_c as a function of pressure. Figure 6 shows the trend of χ and χ_c with pressure at 40 and 80°C and the common intercepts of the two equations. The χ parameters intersect at 110 and 225 atm for 40 and 80°C, respectively. Both sets of experimental miscibility pressures are designated by arrows on the pressure axis in Figure 6. These values are in excellent agreement with the extrapolated pressures quoted above and independent experimental data determined by Stahl (47,48).
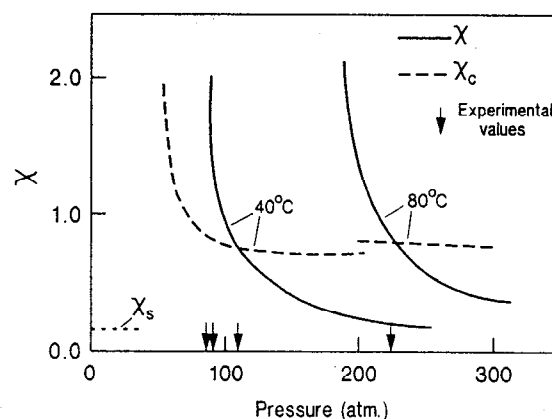

Figure 5. Solubility of triglycerides in supercritical CO_2 as a function of temperature and pressure.

Figure 6. Prediction of miscibility for triglycerides in supercritical CO_2.

It should be noted that the assumed χ_s value shown in Figure 6 is 0.167, a value associated with an infinite linear chain configuration in a solvent (49). However, the magnitude of χ_s, which normally ranges between 0.1 and 0.6, has a limited influence on the derived miscibility pressure in Figure 6. For example, if one assumes that χ_s is zero, a value normally associated with a very large polymer, the intercept-derived miscibility pressure is approximately 90 atm. This value is within the limits of quoted experimentally derived miscibility pressures (47) and therefore does not invalidate the theoretical approach offered here.

The above theoretical concepts can also be used to predict the differential extraction of one solute with respect to another as the extraction pressure is raised. Figure 7 is a graph of χ and χ_c versus pressure for the DDT–supercritical CO_2 system at 60°C. Here, χ_c intercepts the loci at 180 atm, indicating that DDT should be dissolved in the supercritical fluid phase at this degree of gas compression. The results from extraction experiments performed on a 100-ppm spike of DDT in lard at approximately 100 atm intervals are shown in Table II. At 95 atm, there was a negligible yield of the lipid background matrix and only a trace of DDT was detected because of the use of an electron capture detector, which is specific for chlorinated pesticides. Increasing the extraction pressure to 204 atm resulted in a finite yield of fat and a concentration of 585 ppm of the pesticide in the lipid matrix. This represented about 75% of the original spike level in the lard sample. Further extraction of this sample at a pressure approaching 300 atm yielded a significant
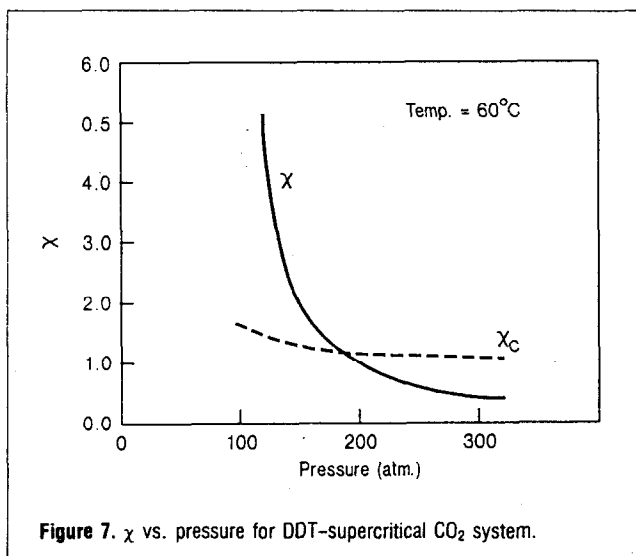
quantity of triglycerides and 9 ppm of additional pesticide. Clearly, the enrichment of the pesticide in the extract between 100 and 200 atm is consistent with the predictions shown in Figure 7 and offers a way of enriching the desired analyte in an extract for subsequent analysis. Similar results have been recently obtained for the SFE of polychlorinated biphenyls in fish oil by Krukonis (50).

The results in Table II also suggest that it may be possible to isolate very low levels of analytes from interfering matrix components by precisely controlling the extraction pressure. For example, it should be possible to extract most of the DDT from lipid interferences if the extraction is performed at approximately 100 atm. Extraction of trace pesticide levels has recently been demonstrated by Kapila and co-workers (51) for chlorinated pesticides at the ppb level in a fish lipid matrix. The relative proportion of lipid or analyte in the extract is governed by the differences in their respective miscibility pressures; therefore, the appearance of considerable lipid in the 204 atm extract in Table II is consistent with the trends portrayed in Figure 5. Many compounds exhibit subtle differences in their miscibility pressures in the compression region of 100–200 atm; however, it takes precise control of the extraction pressure to facilitate enrichment of one component from another.

The differential pesticide concentrations reported as a function of extraction pressure in Table II are in part due to their low solubilities in supercritical CO_2 in this experimental pressure range. More rapid and complete extractions of particular analytes are possible by employing higher pressures, thereby increasing the analyte concentration per unit of time in the flowing fluid phase. Unfortunately, at higher extraction pressures, more interfering components become solubilized in the supercritical fluid phase and the resultant extract may require additional purification before further analysis can be performed.

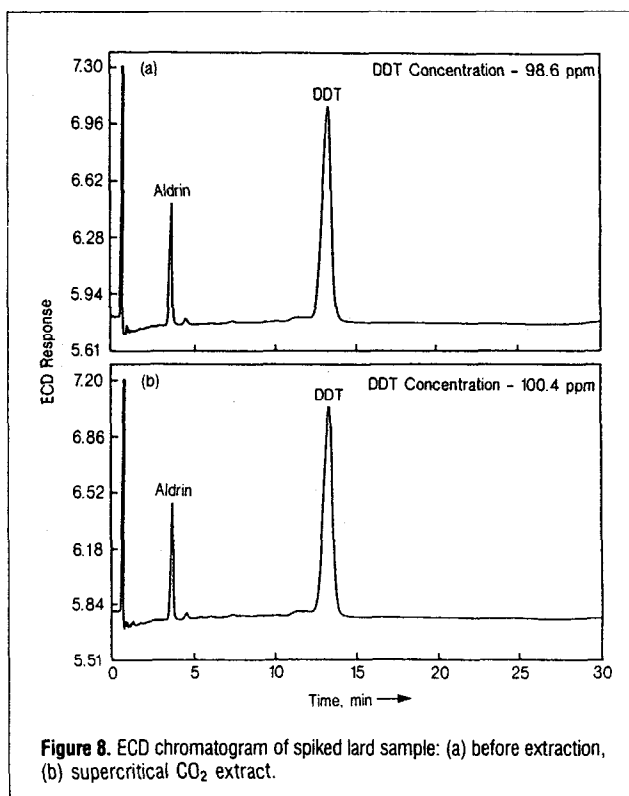Examples of the results that can be obtained by this mode

Figure 7. χ vs. pressure for DDT–supercritical CO_2 system.

Table II. Effect of Pressure on DDT/Fat Extraction*

Pressure	Fat yield (g)	DDT concn (ppm)	DDT wt (g)	% DDT
1400 psig (95.2 atm)	0	13.7	0	0
3000 psig (204 atm)	2.57	585	0.0015	75.2
4200 psig (296 atm)	13.85	12.9	0.000178	8.93

* Conditions: 20 g of 100-ppm spiked lard; temperature, 60°C; CO_2 flow, ~4.0 SLPM.

Figure 8. ECD chromatogram of spiked lard sample: (a) before extraction, (b) supercritical CO_2 extract.

of extraction are shown in Figures 8a, 8b, and 9. A 100-ppm spiked lard sample (actually assayed at 98.6 ppm) was extracted at 5000 psig (340 atm) and 60°C on the fabricated extractor described in the experimental section; the DDT was recovered at a 100.4-ppm level in the total lipid extract. Note that the resultant chromatogram (Figure 8b) of the supercritical-fluid-extracted sample is identical to the original spiked sample (Figure 8a) (aldrin is added as an internal standard during the ECD-GC assay), indicating that the SFE did not introduce any additional artifacts that the sample cleanup procedure could not remove. Similarly, a total lipid SFE of six spiked pesticides at 1-10 ppm levels in lard using the Milton Roy extraction unit at 5000 psig and 60°C yielded analyte recoveries above 94%. Once again, the SFE with CO_2 yielded an extract free from interferences after the sample cleanup procedure was applied, as shown by the artifact-free ECD-GC profile in Figure 9.

As noted in the introduction, there are specific cases in which SFE can be performed in situ during an SFC analysis. The principal requirement for performing such an operation is that all of the components in the sample matrix can be solubilized in the supercritical fluid mobile phase. This approach obviously has some advantages, because sample preparation by conventional methods or SFE, either on-line or off-line, is avoided. Figure 10 shows an example of this method as applied to the 100-ppm DDT spiked lard sample. Here an Alcoa alumina packed microbore column (100 × 1 mm) was used in conjunction with a supercritical CO_2 mobile phase, pressure programmed under the conditions specified in the figure. Neat injection of the undiluted lard sample and selective solubilization of the pesticide and fat moieties into the supercritical fluid mobile phase yielded the separation in Figure 10. Although the flame ionization detector (FID) response is low at the stated pesticide concentration levels, the concept of separating the background matrix from the analyte of interest is demonstrated. Increasing the sensitivity of the FID has permitted levels of DDT between 1 and 10 ppm to be detected. Recently, several research groups (52,53) have had some success using the above analysis principle with electron capture detection.

The above experimental results suggest that the theoretical concepts advocated earlier may have some applicability for predicting the mobile phase pressures or densities required for fractionating solutes in SFC. Many experimental factors besides the mobile phase pressure or density impact on solute retention in SFC, including the interaction of the solute with the stationary phase, the mobile phase interaction with the stationary phase, and the rate of mobile phase pressure or density change as a function of time. Such factors, however, are relatively minor when compared to the selective solvent power produced by pressurization of the supercritical fluid mobile phase, particularly when one is concerned with separating compounds of similar molecular structure, such as oligomers.

Klesper and Hartmann (54) demonstrated several years ago that fractionation of polymeric mixtures requires certain threshold pressures to solubilize oligomeric fractions of synthetic polymers into the SFC mobile phase. Therefore, a predictive method that allows an estimate of these required threshold miscibility pressures from solute and solvent physical property data will be of considerable value. In addition, if the SFC separation is controlled by selective solubilization of the oligomeric species into the compressed fluid phase, then the predictions offered by the above theoretical approach could define a pressure or density interval over which selective fractionation of the mixture would be possible.

To test this hypothesis, we have applied our theoretical concepts to literature results on the SFC separation of oligomeric mixtures. Programmed pressure and density chromatographic separations were chosen to represent a wide variation in chromatographic conditions, including experimental pressure and temperature, type of oligomeric solute, and chromatographic columns. Our predictive method was applied primarily to homopolymers, mobile phases containing no modifiers, and separations performed under isothermal conditions. In addition, the effect of χ_s was assumed to be minimal over the quoted pressure ranges and relatively low reduced temperatures. Therefore only the enthalpic contribution to χ, χ_H, was used in the subsequent calculations. Experimental pressure fractionation ranges were estimated from the pressures and densities at which the oligomeric solutes eluted from the chromatographic column. These were calculated from the actual chromatograms by interpolating the elution peak position to the pressure or density value given on the elution time axis. Requisite solubility parameters and molar volumes for the initial and terminal oligomer species were estimated from the method of Fedors (36).

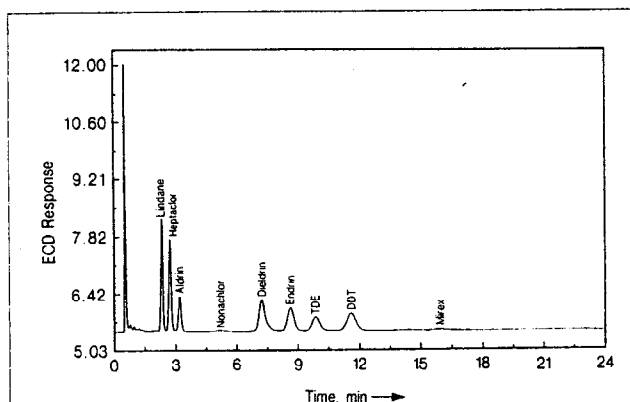Figure 11 is a plot of χ_H versus pressure, covering the con-

Figure 9. ECD-GC profile of spiked lard extract after supercritical CO_2 extraction.
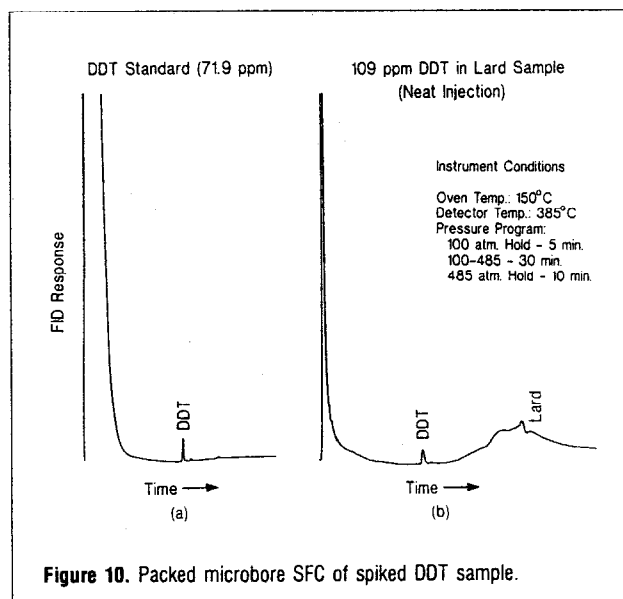
Figure 10. Packed microbore SFC of spiked DDT sample.

ditions used for the capillary SFC separation of n-alkane oligomers ranging from C_{18} to C_{30} at 40°C on a cross-linked SE-54 stationary phase (55). In order to define the fractionation range pressures, two individual curves must be constructed for the C_{18} and C_{30} oligomers as well as for the χ_H plots of these respective solutes. The miscibility pressures for these respective solutes are then defined by the intersection points of each χ_H line with its corresponding χ_c plot and their relative positions on the pressure axis. For the case of the n-alkane oligomers, the experimental elution pressures were 91 and 105 atm for the C_{18} and C_{30} oligomers, respectively. These values are in excellent agreement with the extrapolated values defined by the intersection points of the χ_H and χ_c plots, which are designated by the arrows on the pressure axis in Figure 11. Note that the individual χ_H lines are very close together over the pressure range specified in Figure 11. This trend results in their intercept values with respective χ_c plots being of similar magnitudes. Therefore, one would need very precise control over the density or pressure gradient in SFC to effect separation of this particularly oligomeric mixture. Fortunately, such chromatographic equipment is available.

A similar plot applicable to the case of SFC fractionation of polymethylsiloxane (PMS) oligomers is shown in Figure 12. Here chromatographic fractionation was effected at 40°C over a very narrow pressure range, 93–103 atm for oligomers from $n = 10$ to $n = 40$. The predictions offered by the intercepts of the χ_H and χ_c plots for the above oligomer range are shown by the arrows on the pressure axis. These values of 89 and 97 atm respectively are in excellent agreement with the above experimental values defined by the SFC separation (56).

Expansion of the above theoretical concept to higher chromatographic fractionation temperatures and a more polar oligomeric mixture are shown in Figures 13 and 14. For the case of a capillary SFC fractionation of C_{30} to C_{70} hydrocarbons at 160°C on a DB-5 coated fused-silica column (57), the theoretical predictions, designated by the arrows on the pressure axis, fall at the midpoint of the experimentally recorded elution pressures. Although the agreement between theory and experiment is not as satisfactory in this case as in the previous examples, it still provides a useful prediction of the pressure range in which to

start a chromatographic fractionation, as well as conditions amenable to the solubilization of the solutes. Similar results are also obtained for the system Triton X-100 and supercritical CO_2. Here the theoretical predictions fall within the experimental fractionation ranges recorded in the literature (58,59), as shown in Figure 14. In this case, note that only a small error is introduced into the estimation of the miscibility pressure for the $n = 17$ oligomer by neglecting χ_s.

Conclusions

Supercritical fluid extraction can be utilized in a variety of modes for sample preparation and chromatographic separations, depending on the desired result. This paper has illustrated some of the potential methods for using SFE to the analyst's advantage. A simple theory, based on the Hildebrand solubility parameter theory and the Flory–Huggins χ parameter, has been shown to have predictive value for optimizing extraction and fractionation conditions. Theoretical predictions utilizing this theory have been in good agreement with experimental results for the extraction of total lipid matter from sample matrices
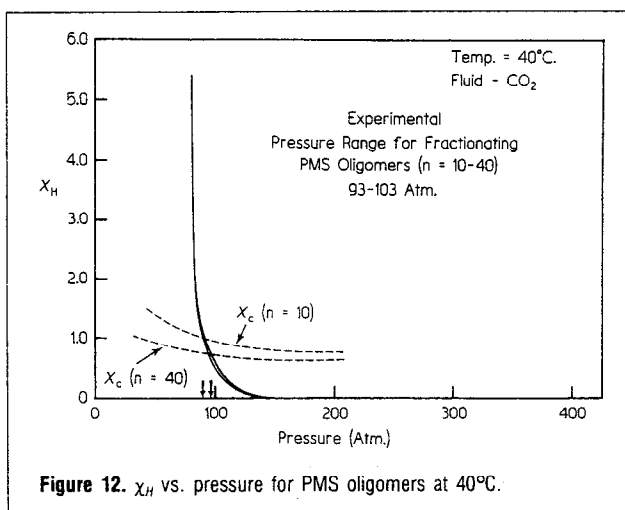
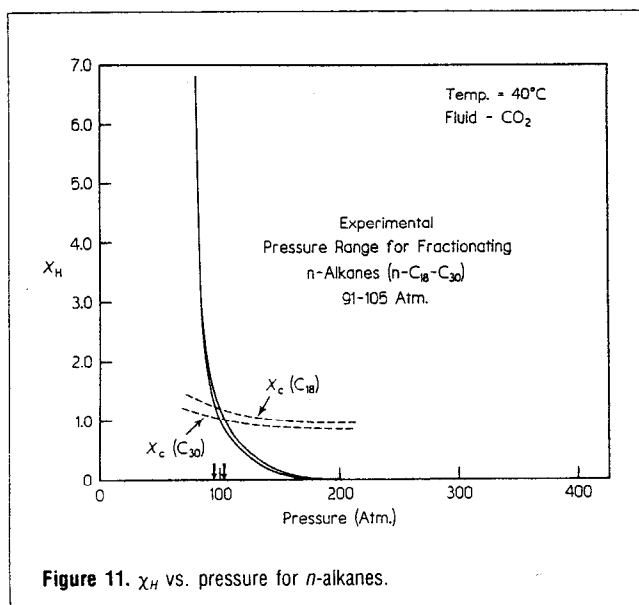**Figure 12.** χ_H vs. pressure for PMS oligomers at 40°C.
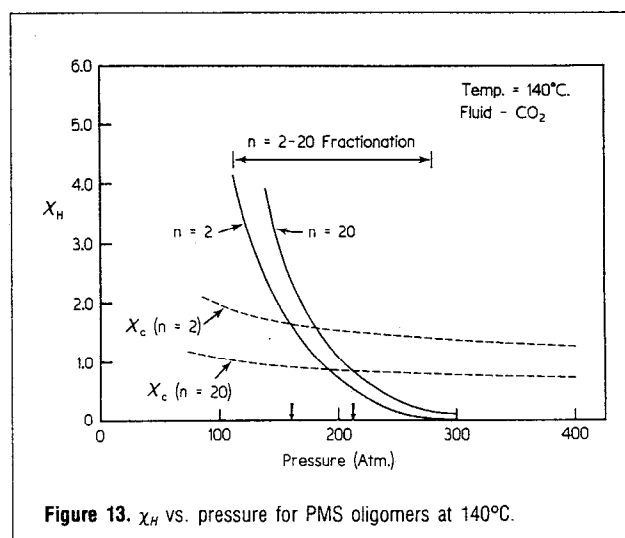
Figure 11. χ_H vs. pressure for n-alkanes.

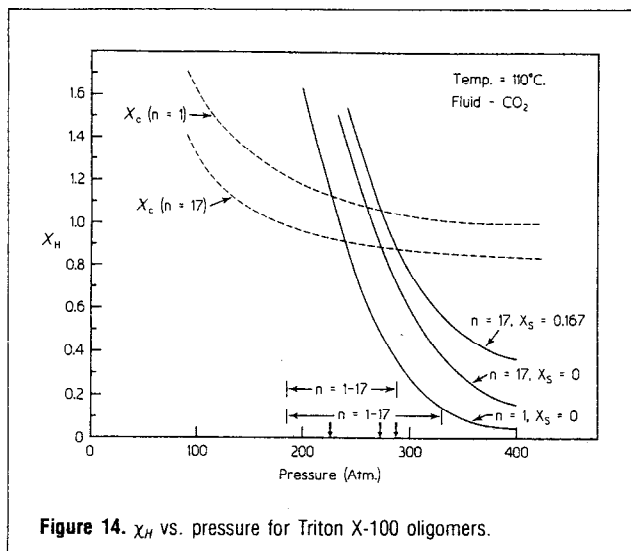**Figure 13.** χ_H vs. pressure for PMS oligomers at 140°C.

Figure 14. χ_H vs. pressure for Triton X-100 oligomers.

and the separation of pesticides from interfering components. Results of this simple predictive method, which requires a minimum of physical and structural data for application, also have shown promise in specifying conditions for SFC separation of oligomeric mixtures.

Disclaimer

The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

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